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Effect of cooking on proteinase inhibitors of *Dolichos* lablab bean (*Dolichos lablab perpureus* L.)

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Abstract. Proteinase inhibitory activity in ten different varieties of *Dolichos lablab perpureus.* L. was determined. All the varieties tested exhibited appreciable level of proteinase inhibitory activity (PIA). The trypsin inhibitory activity (TIA) (Mean: 20170 TIU/g) was relatively higher than the chymotrypsin inhibitory activity (CIA) (Mean: 15380 CIU/g). Effect of temperature and cooking on PIA was studied. The nature of cooking medium and duration of cooking had profound effect on the PIA. The dry fried seeds lost their PIA very rapidly (91% in 20 min). Seeds cooked in slightly alkaline medium lost their PIA quickly (89% in 30 min) compared to those cooked in acidic (80% in 30 min) and neutral pH (83% in 30 min). The PIA in green pods was also determined and they had only one third of the PIA (8200 TIU/g and 8125 CIU/g) found in the dry seeds.

Introduction

Protein proteinase inhibitors are widely distributed in plants and microbial systems [1–3]. Since early 1940s, the plant inhibitors have been extensively studied as antinutritional factors due to the potential adverse effects they produce on human and animal intestinal tracts [1, 2, 4]. The legumes have provided large amount of protein in the human diet. However, their use as sole sources of protein has been subdued due to the presence of the antinutritional factors, in particular, the proteinase inhibitors. Nevertheless, there has been a remarkable improvement in the nutritional quality of legume foods due to various treatment methods [5, 6].

Dolichos lablab perpureus is extensively grown in India [7]. In South India, the seeds are consumed as whole seeds as well as split pulse. Generally, the seeds are cooked with spices or dry fried. The proteinase inhibitors of *Dolichos lablab perpureus* have been partially characterized [8, 9] and have been shown to inhibit human trypsin and chymotrypsin [4, 10]. Many proteinase inhibitors are resistant to heat and acidic pH [11–13]. This heat stability of proteinase inhibitors and their proved adverse effects on digestive tracts prompted us to study the effect of cooking on proteinase inhibitors of *Dolichos lablab perpureus* seeds.

Screening of different varieties of *Dolichos lablab perpureus* has also been carried out to ascertain the variation in the PIA level with reference to the genetic variation.

Materials and methods

Samples. Different varieties of field bean seeds were procured from Germplasm Collection, Pulse Management Department, University of Agricultural Science, G.K.V.K., Bangalore 65, India.

Chemicals. Casein (technical grade), trypsin (IX crystalline, salt free, DPCC-treated type IX), chymotrypsin (3X crystallised, salt free) were purchased from Sigma Chemical (St Louis, USA). All other chemicals were of analytical grade.

Frying. Dry seeds were fried on a frying pan for 20–25 min.

Cooking. 2 g seeds were cooked in a boiling water bath (97 °C) for different time intervals – 10, 20, 30 and 60 min in 50 ml water of varying pH (5.7, 7.0, 8.6) and salt (pH 7.0 with 0.2 M NaCl). The pH of the water in each case was adjusted by using dilute acetic acid or ammonium hydroxide.

Heat treatments. 10 ml aliquots of inhibitor extract from dry seeds were adjusted to acid (3.0), alkaline (12.0) and neutral (7.0) pH with dilute acetic acid and ammonium hydroxide. The pH adjusted aliquots were subjected to heating at different temperatures ranging from 30 to 90 °C for 30 min, the extracts were then cooled in ice-bath and residual TIA and CIA were determined after adjusting the pH to 7.6.

Extraction of proteinase inhibitors. Dry seeds and fried seeds: whole seeds were ground to fine powder and defatted with acetone. The defatted flour was extracted with 100 mM phosphate buffer pH 7.6 for 4 hours at 4 °C by taking flour to buffer ratio of 1:10 (w/v). The suspension was centrifuged for 30 min at 10,000 rpm at 4 °C and the clear supernatant was used for estimation of PIA and protein.

Cooked seeds: seeds cooked for different time intervals were homogenised with phosphate buffer in an all glass pottor Elvejhem type homogenizer. The homogenate was centrifuged at 10,000 rpm for 30 min and the supernatant was used for estimation of PIA and protein.

Assay of proteinases. The trypsin and chymotrypsin activities were determined by the caseinolytic method [14, 15]. 1 ml of phosphate buffer, pH 7.6 containing 25 μg of trypsin or 20 μg of chymotrypsin was incubated at 37 °C with 1.0 mL 1% casein in phosphate buffer or borate buffer, pH 7.6 for 20 and 10 min respectively. The reaction was arrested by adding 3 ml of trichloroacetic

acid. The suspension was filtered and the absorbance of the filtrate was read at 280 and 275 nm for trypsin and chymotrypsin respectively in UV-VIS specord (Carl Zeiss). Estimation of TIA and CIA was carried out by incubating different aliquots of the inhibitor extract with 25 μg of trypsin and 20 μg of chymotrypsin for 15 min, and the residual activities of the enzymes were determined by the caseinolytic method.

Units. One unit of trypsin and chymotrypsin is defined as 0.01 increase in the absorbance at 280 nm and 275 nm respectively under the assay condition. The trypsin inhibitory units (TIUs) and chymotrypsin inhibitory units (CIs) are the number of trypsin and chymotrypsin units inhibited under the same assay conditions.

Results and discussion

Proteinase inhibitory activity (PIA) in different varieties of Dolichos lablab perpureus. The PIA estimated in different varieties of Dolichos lablab bean seeds are summarized in the Table 1. All the varieties tested had good amount of PIA. The level of TIA was marginally higher than the level of CIA. These PIAs are comparable to those of red kidney bean (*Phaseolus vulgaris*) and horse gram (*Macrotyloma uniflorum*) [11, 16]. However, the levels were higher than those reported for finger millet (*Eleusine Coracana*) [13]. The variation in the PIA levels in different varieties may be due to the genetic variation in the varieties as observed in the case of finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum typhoideum*) [13, 17].

Table 1. Proteinase inhibitory activities in *Dolichos lablab* varieties

	Variety	Seed colour	TIU/g	CIU/g	Soluble protein mg/g
1.	HA-3	Brown	23,000	20,650	146.0
2.	Maniavare	Brown	22,750	19,100	133.0
3.	Race-21	Black	27,700	16,300	74.0
4.	Race-31	Cream	19,200	10,800	73.0
5.	Race-42	Reddish Brown	11,750	8,000	177.0
6.	Race-54	Black	11,800	11,150	175.0
7.	Race-62	Brown	23,900	16,300	190.0
8.	Typicus	Brown	26,900	24,300	167.0
9.	Typicus	Black	13,700	11,250	120.0
10.	G.K. avare	Cream	21,000	16,000	130.0
	Average		20,170	15,380	138.0
11.	Green pods of maniavare		8,200	8,100	102.0

The *Dolichos lablab perpureus typicus* which is a garden variety, whose pods are mainly consumed as vegetable had highest TIA and CIA. The lower levels of PIA in green seeds (maniavare) is in conformity with the observation of Ambe & Sohanie [18], that proteinase inhibitors are synthesized at later stages of the seed development, and that their physiological role is as storage proteins. It is noteworthy that the newly developed variety (HA-3) which is replacing the traditional varieties for season independent cultivation and higher yields, exhibited higher PIA than the traditional ones.

Effect of heat and cooking on proteinase inhibitors of Dolichos lablab perpureus. Effect of heat on proteinase inhibitors in crude extracts showed their stability to heat at acid and neutral pH and their relative lability at alkaline pH (Table 2).

The effect of heat treatment on proteinase inhibitors in crude extracts are entirely different from cooking conditions, involving whole seeds. Hence, it was imperative to study the effect of cooking on PIA on whole seeds. Table 3 shows the effect of cooking on proteinase inhibitors of *Dolichos lablab perpureus.* The PIA was completely lost by 60 min cooking in all the cooking conditions chosen. Cooking for 10 min had moderate effect on PIA except in pH 8.5 (40% loss of TIA). Nearly 50% of PIA was lost after 20 min of cooking in all the chosen conditions. The residual CIA and TIA after 30 min cooking appeared to be the same in all the conditions (16–19%). Comparison of 30 min heating data with that of 30 min cooking indicated that PIA is more rapidly lost in crude extracts than in whole seeds except in acidic pH where both CIA and TIA were highly stable (80% CIA, and 97% TIA).

The dry frying for 20 min resulted in remarkable denaturation of PIA. Though dry frying, appeared to be effective in denaturing the proteins inhibitors, the soluble protein content was very low when compared to 30 min cooked seeds which are ideal for consumption (Table 3).

Although cooking methods seem to be irrational in rural parts of South

Table 2. Effect of temperature on *Dolichos lablab* inhibitors

Temperature (°C)	Inhibitory activity					
	TIA (%)			CIA (%)		
pH	3.0	7.0	12.0	3.0	7.0	12
25	100.0	100.0	100.0	100.0	100.0	100.0
40	100.0	92.8	97.4	94.4	98.5	81.5
50	100.0	89.2	82.7	90.7	96.8	67.6
60	97.0	88.6	12.8	91.6	95.6	17.6
70	97.0	90.2	7.7	86.1	94.6	12.0
80	97.0	68.0	6.9	88.8	80.1	15.7
97	97.4	8.2	7.7	80.5	4.66	16.6

CIA = chymotrypsin inhibitory activity; TIA = trypsin inhibitory activity.

Table 3. Effect of cooking on proteinase inhibitory activity of *Dolichos lablab perpureus* L. seeds

Duration of cooking	Cooking conditions				Dry frying
pH	7.0	5.7	8.5	7.0 & NaCl	7.0
Trypsin inhibitory units					
Dry seeds	22000				
10 min	20460	21340	13300	21560	
20 min	10400	11440	6760	11336	1992
30 min	3840	4240	2518	4204	
60 min	000	000	000	000	
Chymotrypsin inhibitory units					
Dry seeds	20500				
10 min	20000	17940	15200	13060	
20 min	12400	11360	9424	8085	1503
30 min	4345	3900	3308	2834	
60 min	000	000	000	000	
Soluble protein (in mg)					
Dry seeds	144				
10 min	133	125	131	137	
20 min	109	100	107	112	13.5
30 min	64	70	68	63	
60 min	31	38	63	34	

India, these results indicate that they are effective in destruction of antinutritional factors (PI) in the *Dolichos lablab perpureus.* It can be surmised that nature of cooking medium such as pH of water, salt content and duration of cooking medium such as pH of water, salt content and duration of cooking are important factors to be considered for preparation of dishes of this bean, as these conditions vary depending on the geographical locations.

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